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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Srivastava et al.	Confirmation No.:	7769
Serial No.:	09/750,972	Art Unit:	1642
Filed:	December 28, 2000	Examiner:	Christopher H. Yaen
For:	ALPHA (2) MACROGLOBULIN RECEPTOR AS A HEAT SHOCK PROTEIN RECEPTOR AND USES THEREOF	Attorney Docket No:	8449-134

DECLARATION OF PRAMOD K. SRIVASTAVA UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, PRAMOD K. SRIVASTAVA, do declare and state that:

1. I am a citizen of India, and a permanent resident of the United States residing at 70 Pheasant Run, Avon, Connecticut 06001.

2. I am a co-inventor with Robert J. Binder of the invention described and claimed in the above-identified patent application, Application No. 09/750,972 ("the '972 application"). I am a co-founder and shareholder of Antigenics, Inc., exclusive licensee of the above-identified application.

3. I am currently Professor of Immunology and Director of the Center for Immunotherapy of Cancer and Infectious Diseases at the University of Connecticut School of Medicine, the position I have held from January 1997 to the present. The University of Connecticut Health Center is assignee of the above-identified application. From 1993 to December 1996, I was a member of the Department of Biological Sciences at Fordham University, Bronx, New York where I served as Professor and Head of the Cancer Immunology Program.

4. My academic and technical experience and honors, and a list of my

publications are set forth in my curriculum vitae, which is attached hereto as Appendix 1.

5. I have read and am familiar with the '972 application. The '972 application teaches that the alpha (2) macroglobulin receptor serves as a cellular receptor for HSPs and HSP-peptide complexes. The '972 application discloses the use of alpha (2) macroglobulin ("α2M") receptor (also referred to as the CD91 receptor) as a heat shock protein receptor, the use of modified cells that express the α2M receptor bound to an HSP, and the use of anti-CD91 antibodies and other molecules that bind the α2M receptor-HSP complex. The '972 application also discloses the use of molecules, such as antibodies, that interfere with HSP-alpha (2) macroglobulin receptor interactions, for treating and preventing autoimmune disorders, proliferative disorders, and infectious disease. As described in the '972 application, in certain embodiments, an anti-CD91 antibody can effectively inhibit re-presentation of peptides chaperoned by heat shock proteins (HSPs) *in vitro*.

6. I have read and am familiar with the pending claims and the outstanding Office Action dated December 4, 2002 for the '972 application. I have been informed and believe that the claims of the '972 application are subject to a rejection based on the contention that the '972 application does not provide sufficient guidance for the use of anti-CD91 antibodies for treating or preventing autoimmune disorders, and that such treatment or preventative methods would require undue experimentation.

7. The following experiments were conducted by me or under my supervision at the Center for Immunotherapy of Cancer and Infectious Diseases at the University of Connecticut School of Medicine. The first set of experiments described below show that anti-CD91 antibodies effectively inhibit re-presentation of peptides chaperoned by HSPs via an HSP-alpha (2) macroglobulin receptor interaction in normal mice. The results demonstrate that an anti-CD91 antibody can effectively interfere with HSP-alpha (2) macroglobulin receptor function *in vivo* and suppress an immune response. The second set of experiments describes successful experiments wherein mice were injected with anti-CD91 antibodies and complexes of HSP and tumor-specific peptides, followed by tumor challenge. The results demonstrate that anti-CD91 antibody effectively inhibits re-presentation of the tumor-specific peptides and suppresses an immune response, resulting in uncontrolled tumor growth, in comparison to mice injected with the complexes alone, which exhibited tumor protection. The third set of experiments describes successful results which demonstrate that injection with alpha (2) macroglobulin polypeptide, an antagonist of the interaction between HSP and alpha (2) macroglobulin receptor, inhibits the onset of autoimmune disorder in non-

obese diabetic mice (NOD), demonstrating that alpha (2) macroglobulin can suppress autoimmune damage and delay the onset, *i.e.*, prevent, diabetes in NOD mice. Taken together, these results are supportive of and predict the ability of an anti-CD91 antibody to interfere effectively with an HSP-alpha (2) macroglobulin receptor interaction to suppress an immune response *in vivo*, thereby treating an autoimmune disorder.

8. The first set of experiments were *in vivo* re-presentation assays which demonstrated that an anti-CD91 antibody or alpha (2) macroglobulin can effectively inhibit re-presentation of OVA20 peptides chaperoned by a heat shock protein, gp96. The *in vivo* re-presentation assays involved injecting mice with gp96-OVA20 peptide complexes, which allowed for re-presentation of chaperoned peptides to occur. Re-presentation of peptides was then detectable in dendritic cells of the draining lymph nodes by staining lymph node extracts with anti-OVA20 antibody (further details of the experimental procedures and methods provided in Appendix 3).

9. In the experiment shown in Figure 1 (see Appendix 2) non-covalent complexes of gp96 and ovalbumin peptide (gp96-OVA20) were co-administered (as indicated by the horizontal line below the X-axis) with either phosphate buffer saline (PBS), anti-CD91 antibody (CABEL IgG), control IgG (Rabbit IgG), albumin, or mouse alpha (2) macroglobulin (α2M). PBS alone was administered as a negative control. The first set of data points shows that gp96-OVA20 complex co-administered with PBS did not inhibit re-presentation of OVA20 peptide (control). The second set of data points shows gp96-OVA20 complex co-administered with CABEL effectively inhibited re-presentation of OVA20 peptide, exhibiting nearly a five fold difference in average percent positive cells in comparison to the control. The third set of data points shows that gp96-OVA20 complex co-administered with control rabbit IgG did not effectively inhibit re-presentation on OVA20 peptide. Likewise, gp96-OVA20 complex co-administered with albumin did not effectively inhibit re-presentation on OVA20 peptide, as indicated in the fourth set of data points. The fifth set of data points shows gp96-OVA20 complex co-administered with alpha (2) macroglobulin nearly completely inhibited re-presentation on OVA20 peptide. The sixth set of data points shows that no signal was detected when control PBS alone, without co-administration of gp96-OVA20 was used. These results demonstrate that anti-CD91 antibodies inhibit re-presentation of heat shock protein chaperoned peptides *in vivo*. The results also predict that an anti-CD91 antibody can effectively interfere with an HSP-alpha (2) macroglobulin receptor interaction and down-regulate an immune response *in vivo*, since

re-presentation of OVA20 peptide on the surface of dendritic cells of the lymph nodes, should result in T cell activation and an immune response.

10. For the second set of experiments, C57BL/6 mice were challenged with live tumor cells expressing OVA20 peptide at 14 and 7 days after co-administration of anti-CD91 antibody and gp96-OVA20 complex, and tumor volume was monitored. A control group of C57BL/6 mice were challenged with live tumor cells expressing OVA20 peptide at 14 and 7 days after co-administration of rabbit IgG and gp96-OVA20 complex, and tumor volume was monitored. In this assay, re-presentation of OVA20 peptide generated an immune response that prevents tumor growth, whereas administration of anti-CD91 antibody suppressed re-presentation of OVA20 peptide, allowing growth of tumors. Rapid and progressive tumor growth was indicative of suppression of re-presentation of OVA20 peptide by the anti-CD91 antibody (further details of the experimental procedures and methods are provided in Appendix 3). Figure 2 of Appendix 2 (bottom) shows that co-administration of anti-CD91 antibodies and non-covalent complexes of gp96 and OVA20 inhibits representation of OVA20 peptide and effectively suppresses an immune response to tumor challenge *in vivo,* where the tumor cells express OVA20 on their cell surfaces. The results demonstrate that an anti-CD91 antibody can effectively interfere with an HSP-alpha (2) macroglobulin receptor interaction and down regulate an immune response.

11. The third set of experiments utilized the NOD mouse model, a mouse model for diabetes, an autoimmune disorder, in humans. NOD mice develop diabetes as a result of autoreactive T cell destruction of the beta cells of the pancreas. The incidence rates of diabetes in these mice is well established in published literature, wherein it is reported that over 80% of female mice developed diabetes by 24 weeks of age and onset of insulitis commenced between 6-8 weeks of age. NOD mice are inbred and highly responsive to a variety of immunoregulatory strategies.

12. The effect of varying doses of alpha (2) macroglobulin was investigated in female/LtJ NOD mice over a time period during which symptoms of diabetes are known to develop in untreated animals. NOD mice receiving no treatment served as controls. Activated alpha (2) macroglobulin doses compared included 700 μg, 70 μg, and 7 μg administered at week 9 and again at week 10. Mice receiving the activated-albumin control were administered 700 μg doses at week 9 and again at week 10. Further details of the experimental procedures and methods are provided in Appendix 3. As shown in Figure 3 (see Appendix 2), the mice administered the 70 μg doses of activated alpha (2) macroglobulin

did not develop diabetes until the 13th week of life and mice administered the 7 μg and 700 μg doses did not develop diabetes until the 14th week of life, 1-3 weeks later than control animals developed diabetes. The percent incidence was based on urine glucose levels that are known to be indicative of diabetes. The results demonstrate that alpha (2) macroglobulin can suppress autoimmune damage of pancreatic cells that leads to the increased urine glucose levels indicative of diabetes. These results demonstrate that alpha (2) macroglobulin can delay the onset of diabetes in NOD mice. The results also show that mice injected with 700 μg of alpha (2) macroglobulin have only a 40% incidence of disease at the end of the study (week 20) in comparison to non treated mice and albumin control mice, which had 80% incidence of disease. This is predictive of the efficacy of treatment methods using antagonists of alpha (2) macroglobulin in humans having autoimmune disease.

13. In view of the foregoing, I conclude, and others skilled in the art would also conclude, that anti-CD91 antibodies are capable of suppressing immune responses against HSP chaperoned peptides *in vivo*, and predicts the efficacy of anti-CD91 antibodies *in vivo* to suppress an immune response and thereby treat and prevent an autoimmune disorder.

14. I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 6/9/03

PRAMOD K. SRIVASTAVA

Attachments:

Appendix 1: *Curriculum Vitae* of Pramod K. Srivastava

Appendix 2: Figures 1 to 3.

Figure 1: The role of CD91-gp96 interaction in *in vivo* re-presentation.

Figure 2: Suppression of immunity is dependent on CD91 antibodies.

Figure 3: A CD91 ligand prevents autoimmune diabetes in NOD mice.

Appendix 3 Materials and methods for experiments

Curriculum Vitae of

Pramod K. Srivastava

Name: Pramod Kumar SRIVASTAVA

Date of Birth: August 16, 1955

Nationality: Permanent Resident of USA

Social Security #: 043-74-4345

Home address: 70 Pheasant Run
Avon, CT 06001

Office Address: Center for Immunotherapy of Cancer & Infectious Diseases
University of Connecticut School of Medicine, MC1601
Farmington, CT 06030 - 1601

Tel: 860 679 4444
Fax: 860 679 7905
E-mail: srivastava@nso2.uchc.edu

Education:

1973, B Sc (Zoology, Botany and Chemistry), Allahabad Univ, Allahabad, India, High Second div.;

1975, M Sc (Botany, with specialization in Palaentology, Advisor : Professor Divya Darshan Pant), Allahabad University, Allahabad, India, First Division with First Rank;

1979, Post-Graduate Diploma (Microbiology & Biotechnology; specialization in yeast genetics, Advisor : Professor Yasuji Oshima), Osaka University, Osaka, Japan;

1984, Ph D (Biochemistry with specialization in tumor immunology, Advisor : Dr. M. Ramchandra Das), Osmania Univ. (Centre for Cellular & Molecular Biology), Hyderabad, India.

2006, MD, University of Connecticut, Farmington, CT

Postdoctoral Training:

Postdoctoral Fellow, Yale University, New Haven, Connecticut, 1982-1983, Advisor: Professor Alan Garen

Research Fellow, Sloan-Kettering Institute for Cancer Research, New York, 1984-85, Advisor: Professor Lloyd J. Old

Positions and Appointments:

Scientist 'C', Center for Cellular & Molecular Biology (CSIR), Hyderabad, India	06.83 - 02.84
Research Associate, Sloan-Kettering Institute for Cancer Research, Immunology Program, New York	01.86 - 10.88
Assistant Professor, Department of Pharmacology, Mount Sinai School, of Medicine, New York. Also appointed in the Dept of Microbiology	11.88 - 08.93
Associate Professor of Immunology, Dept of Biological Sciences, Fordham University, Bronx, NY.	09.93- 01.96
Professor of Immunology, Department of Biological Sciences, Fordham, University, Bronx, NY.	02.96 - 12.96
Professor of Immunology, Physicians Health Service Chair in Cancer Immunology, and Director, Center for Immunotherapy of Cancer and Infectious Diseases, University of Connecticut, School of Medicine, Farmington, CT.	01.97 - now
Director, Cancer Signature Program University of Connecticut, School of Medicine, Farmington, CT.	01.01 - now

Editorial Positions:

Editorial Board, *Tissue Antigens*, 2001-

Section Editor, Inflammation and Immunity Section, *Cell Stress and Chaperones,* 2000 -

Editorial Board, *Cellular Immunology,* 2000 - 2004

Editor, *Cancer Immunity,* 2000 -

Membership of Study Sections and Advisory Councils:

Experimental Immunology study section, NIH, 1994 - 1998;
Scientific Advisory Council, Cancer Research Institute, New York, and Member, Fellowships Committee of the CRI, 1996 - present;
Chairman, Scientific Advisory Board, and member, Board of Directors, Antigenics, LLC., New York, 1995 - present;
Member, Scientific Advisory Board, and member, Board of Directors, Ikonisys Inc., New Haven, 1998 - present;
Howard Hughes Medical Institute Student Fellowships Review Panel, 1999 - 2001;
Member, Steering Committee for Stress Biology, Int'l Union of Biological Science, 2001 - present;
Biotech Advisory Committee, CT Innovations, Rocky Hill, CT, 2001 - present.

Present Grant Support:

NIH/NCI 7/1/00-6/30/03
5R01CA84479-02 $562,792
Receptor for gp96 on Macrophages and Dendritic Cells

Antigenics, L.L.C. 2/12/98-11/15/03
Use of Heat Shock Proteins for the Development of Therapeutic and Prophylactic Vaccines $4,920,641

Lea's Foundation for Leukemia Research 9/1/99-12/31/02
Treatment of indolent B-cell lymphoma and CLL patients with heat shock protein 70 (HSP70) complexed to autologous tumor proteins or to tumor-specific proteins $55,000

The Charles Dana Foundation 12/01/01-11/30/05
Harnessing CD91 ligands for rapid treatments of Infections of unknown antigenicity $500,000

Peer Reviewed Past Grant Support (selected list):

NIH	5RO1-CA44786-09	9/1/87-8/31/97
	Heat Shock Proteins as Tumor Antigens	$755,421
NIH	7RO1-CA64394-04	9/1/97-4/30/99
	Use of HSP70-Peptide Complexes in Specific Immunity	$121,443 per year
Cancer Research Institute		7/1/89-6/30/93
	Investigator Award	$50,000 per year
DARPA (US Army)		
	BAA96024 (Srivastava)	4/9/97-4/8/00
	Heat Shock Protein-Peptide Complexes As Antiviral Agents	$1,540,109
CAP CURE		1/1/98-12/31/98
	Heat Shock Protein Based Prostate Cancer Vaccine Starting from Single Cell or Small Biopsies	$100,000
NIH	5 P50 CA62924-06	01/01/99-12/31/01
	John Hopkins Oncology Center Influence of Random Mutations on the Antigenicity of Colorectal Tumors	$28,356

Teaching Experience:

Teach part of MD/PhD immunology course, Univ. of Connecticut, Farmington, CT, 2002

Teach part of graduate vaccine course at the Univ. of Connecticut, Storrs, CT, April , 2001

Teach part of graduate immunology course (Tumor immunology) at Univ. of Connecticut.

Teach part of Advanced Immunology Course at University of Connecticut.

Taught Summer Undergraduate Intern Program at University of Connecticut, May 1999.

Taught complete graduate and undergraduate immunology courses at Fordham Univ., 1993 - 96.

Teaching Experience (continued):

Part of a graduate course in Biochemical Pharmacology (development of vaccines) (at Mount Sinai), 1988-93.

Part of an Advanced graduate immunology Course (tumor immunology) (at Mount Sinai), 1992-93.

Medical students lectures on Cancer Chemotherapy and Cancer Immunology (at Mount Sinai), 1992-93.

Training Experience (graduate students, post-doctoral fellows etc.):

Thesis advisor to PhD students. Two MD PhD students Robert G Maki and Zihai Li, five straight PhD students: Daniel Levey, Nathalie Blachere, Sreyashi Basu, Robert Binder, and Ping Peng completed their PhD thesis in my laboratory between 1990 and now. Four PhD students Marissa Caudill, Joseph Kovalchin, Guruprasaadh Muralimohan, and Arpana Srinivasan are presently working towards their dissertations.

Seven post-doctoral fellows (Sreyashi Basu, Robert Binder, Oyvind Halaas, Toyoshi Matsutake, Shin Oshima, Ruibo Wang, and Siqing Wang,) presently work in my laboratory.

Eighteen postdoctoral fellows (Anne Altmeyer, Kirstin Anderson, Rajiv Chandawarkar, Anna Feldweg, Michael Heike, Navdeep Jaikaria, Sylvia Janetzki, Stephanie Kespohl, Sumeet Kumar, Kristi McQuade, Clyde Mendonca, Antoine Ménoret, Dirk Schadendorf, Ryuichiro Suto, Yasuaki Tamura, Heiichiro Udono, Mihir Wagh, and Gunner Weidt) have worked in my laboratory between 1988 and now.

Four of my students/trainees (Zihai Li, Antoine Ménoret, Dirk Schadendorf, and Heichiro Udono) have gone on to establish independent laboratories.

Two students, Michael Schneider (1992) and Steven McCoy (1993), worked towards a Westinghouse project and finished as semi-finalists.

A large number of medical students and Sigma Xi summer students have rotated through my laboratory.

Administrative Experience:

At University of Connecticut School of Medicine:

Director, Center for Immunotherapy of Cancer and Infectious Diseases,	1/1/97- now
Director, University of Connecticut Cancer Center	1/1/01- now
Member, Institutional Animal Care and Use Advisor Committee,	3/1/99-now

Administrative Experience (continued):

Member, Steering Committee, UConn General Clinical Research Ctr 12/1/98- 11/30/99
Various Search Committees

At Fordham University: Served as member of:
Institutional Animal Care and Use Committee
Radiation Safety Committee

At Mount Sinai School of Medicine: Served as member of:
Institutional Committee on Special Grants and Fellowships, 1990-1993;
Institutional Advisory Committee to Center for Laboratory Animal Sciences, 1989-93;
Cancer and Generic Lab Committees of Institute for Human Genomic Studies, 1992;
Departmental Graduate Teaching and Safety & Services Committees, 1988-1993;
Director of the Departmental seminar program, 1992-3.

Honors and Awards:
Merit Scholar, Government of India, 1969
Indian Council of Agricultural Research Award in Plant Physiology, 1973
Gold Medal from University of Allahbad, 1975
UNESCO International Studentship in Microbiology, 1978-1979
Senior Fellowship of Indian Council of Scientific and Industrial Research, 1980
John Hans and Edna Alice Old Postdoctoral Fellowship, Cancer Research Institute, NY 1984-1986
First Independent Research Support and Transition Award of NIH, 1987
Irma T. Hirshl Award, 1989
Investigator Award of Cancer Research Institute, New York, 1989.
Mildred Scheel Lecturer at the International Conference on "Hyperthermia in Clinical Oncology", Munich, Germany, 1993
Listed in the International Directory of Distinguished Leadership, American Biographical Inst., 1994
Member, Experimental Immunology study section, NIH, 1994 - 1999.
Member, Scientific Advisory Council, Cancer Research Institute, New York, and Member, Fellowships Committee of the CRI, 1996 - present
Sigma Tau Foundation Lecturer, Rome, Italy, March 1997
UICC (Union Internationale Contre le cancer) Roll of Honor; inducted 1997
Who's Who in Medicine and Healthcare, 2000-2001

Honors and Awards (continued):

Founding Member, Academy of Cancer Immunology, New York
Klaus Irmscher Lecture, Wistar Institute, Philadelphia, PA, 2000

Membership in Societies :
American Association of Immunologists , American Association of Cancer Research International Interest Group in Biorecognition Technology, American Association for Advancement of Science, International Society for Vaccination (Charter member), Cell Stress Society (Life Member)

Publications: See Appendix 1

Patents: See Appendix 2

Organized Conferences: See Appendix 3

Invited Lectures and Talks: See Appendix 4

Personal information :
Born Sultanpur, U.P., India to Babu Mangla Prasad and Tara Devi Srivastava. Married to Jasmine Shah. One child Vasishth Vidyadhar, born 1991.

APPENDIX 1

Publications

(Abstracts are not included)

A. THESES:

Srivastava, P.K.: Gymnosperms of *Glossopleris* flora. M.Sc. Thesis, University of Allahabad, Allahabad, India, 1975

Srivastava, P.K. Cell surfaces during normal and abnormal growth: Purification of a tumor-associated antigen and a tumor-rejection antigen from a rat hepatoma. Ph.D. Thesis, Centre for Cellular and Molecular Biology, Osmania University, Hyderabad, India, 1983.

B. BOOKS:

Srivastava PK (ed.). Cellular Immunity to Cancer. ImmunoMethods Series, Academic Press, 1997.

Srivastava PK Textbook of cancer immunology, John-Wiley, In preparation.

Srivastava PK. (ed) Heat-Shock Protein-Immune System Interactions, Methods, Academic Press, 2002.

C. REVIEWS:

Das MR, Parnaik VK and Srivastava PK. Molecular biology of malignant transformation. Biochemical Reviews. 51: 47-60, 1981.

DeLeo AB and Srivastava PK. Cell surface antigens of chemically induced sarcomas of murine origin. Cancer Surveys. 41: 21-34, 1985.

Srivastava PK and Old LJ. Individually distinct transplantation antigens of chemically induced mouse tumors. Immunology Today. 9: 78-83, 1988. Also see Immunology Today, 10:78 for response to a letter.

Srivastava PK and Maki RG. Stress-Induced proteins as tumor antigens. Current Topics in Microbiology and Immunology 167 : 109 - 124, 1991.

Srivastava PK. Peptide - binding heat shock proteins in the endoplasmic reticulum: Role in immune response to cancer and in antigen presentation. Advances in Cancer Res. 62:153-177, 1993.

C. REVIEWS: (CONTINUED):

Srivastava PK and Udono H. Heat shock proteins in immune response to cancer: The Fourth Paradigm. Experientia 50(11-12): 1054-1060, 1994.

Blachere NE and Srivastava PK. Heat shock protein-based cancer vaccines and related thoughts on immunogenicity of human tumors. Seminars in Immunology 6 : 349-355, 1995.

Srivastava PK and Levey DL. Alterations in T cells of cancer-bearers: whence specificity? Immunology Today, 17 (8): 365-368, 1996.

Srivastava PK, Ménoret A, Basu S, Binder R, McQuade K. Heat shock proteins come of age: Primitive functions acquire new roles in an adaptive world. Immunity (8): 657-665, 1998.

Srivastava PK and Anderson K. Heat, heat shock, heat shock proteins and death: A central link in innate and adaptive immune responses, Immunology Letters , 74, 35-39, 2000.

Basu S and Srivastava PK. Heat shock proteins: the fountainhead of innate and adaptive immune responses. Cell Stress & Chaperones, 5, (5), 443-451, 2000.

Srivastava PK and Amato RJ. Heat shock proteins: The "Swiss Army Knife" Vaccines against cancers and infectious agents. *VACCINE*, 21;19(17-19):2590-7, 2001.

Srivastava PK. Interaction of Heat shock proteins with peptides and antigen presenting cells : Chaperoning of the innate and adaptive immune responses. Annual Reviews of Immunology, Vol. 20, 395-425, 2002.

Srivastava PK. Heat shock proteins in innate and adaptive immunity. Nature Reviews Immunology, Vol. 2, 185-194, 2002.

D. EDITORIALS and CRITICAL COMMENTARIES :

Srivastava, P.K. Protein tumor antigens. Current Opinion in Immunology 3 : 654 - 658, 1991.

Srivastava PK. Heat shock proteins in specific immunotherapy of cancer. Current Opinions in Immunology, 6(5): 728-732, 1994.

Janetzki S and Srivastava PK. Heat shock protein - peptide complexes as therapeutic vaccines against human cancer. Guest Editorial, Clinical Immunotherapeutics, 3: 325-329, 1995.

Srivastava PK. Do human cancers express shared protective antigens? or the necessity of remembrance of things past. Semin Immunol 8(5): 295-302., 1997.

D. EDITORIALS and CRITICAL COMMENTARIES: (CONTINUED)

Srivastava PK. Immunotherapy of human cancer: lessons from mice. Nature Immunology,1 (5), 363-366, 2000.

Li Z, Menoret A, Srivastava PK. Roles of heat-shock proteins in antigen presentation and cross presentation. Current Opinions in Immunology, 14(1), 45-51, 2002.

E. ORIGINAL PEER-REVIEWD PAPERS:

Srivastava,P.K., Harashima S. and Oshima, Y. Formation of two-spored asci interrupted sporulation in Saccharomyces cerevisiae. J. Gen. Microbiol. 123: 29-37, 1981.

Harashima, S., Srivastava, P.K. and Oshima, Y. "Diad Analysis" for linkage studies in yeasts with poor viability. J.Gen.Microbiol.128: 2728-2737, 1982.

Shashikant, C.S., Srivastava, P.K. and Das, M.R. Identification of a DNA polyme-rase associated with the skeletal framework of plasma membrane from a rat hepatoma. Biochem. Biophys. Res. Comm. 114: 571-578, 1983.

Parnaik, V.K., Srivastava, P.K. and Das, M.R. Inhibition of AMV-reverse transcriptase by an RNA-binding protein from plasma membranes of normal and tumor cells. J. Biosciences. 5: 107-116, 1983.

Srivastava, P.K. Harashima S. and Oshima, Y. Two-spored asci produced by interrupted sporulation in yeasts: A novel approach to linkage analysis in yeast. Mol. Gen. Genetics 191:165-166, 1983.

Srivastava, P.K. and Das, M.R. Serologically unique surface antigen of a rat hepatoma is also its tumor-associated transplantation antigen. Int. J. Cancer. 33: 417-422, 1984.

Srivastava, P.K. DeLeo, A.B. and Old, L.J. Tumor rejection antigens of chemically induced sarcomas of inbred mice. Proc. Natl. Acad. Sci., USA 83: 3407-3411, 1986.

Palladino, M.A., Srivastava, P.K., Oettgen, H.F. and DeLeo, A.B. Expression of a shared tumor-specific antigen by two chemically induced BALB/c sarcomas. I. Detection by a cloned
cytotoxic T cell line. Cancer Research 47: 5074-5079, 1987.

Srivastava, P.K., Chen, Y-T and Old, L.J. 5' structural analysis of genes encoding polymorphic antigens of chemically induced tumors. Proc. Natl. Acad. Sci., U.S.A. 84: 3807-3811, 1987.

Srivastava, P.K., Kozak, C., and Old, L.J. Chromosomal localization of the gene encoding murine tumor rejection antigens gp96. Immunogenetics 28: 205-207, 1988.

E. ORIGINAL PEER-REVIEWED PAPERS: (CONTINUED)

Srivastava, P.K. and Old, L.J. Identification of the human homologue of the murine tumor rejection antigen gp96. Cancer Research 49 : 1341-1343, 1989.

Schadendorf, D. Yamaguchi, H., Old, L.J. and Srivastava, P.K. A novel hetermorphic human cell surface alloantigen gp60 defined by a human monoclonal antibody. J. Immunology , 142 : 1621-1625, 1989.

Srivastava, P.K., Rao, W.S. and Bhargava, P.M. A bit of the cell wall is required for regeneration of complete cell walls in yeast. Indian J. of Biochemistry & Biophysics 25 : 601-604, 1988.

Livingston, P.O., Ritter, G., Srivastava, P.K., Padavan. M., Calves, M. J., Oettgen, H. F. and Old, L.J. Characterization of IgG and IgM antibodies induced in melanoma patients by immunization with purified GM2 ganglioside. Cancer Research, 49 : 7045 - 7050, 1989.

Maki, R.G., Old, L.J. and Srivastava, P.K. Human Homologue of Murine Tumor Rejection Antigen Gp96: Analysis of Regulatory and Coding regions and Relationship to Stress-Induced Proteins.Proc. Natl. Acad. Sci. USA 87: 5658 - 5662,1990.

Srivastava PK and Heike M. Tumor - specific immunogenicity of stress - induced proteins : Convergence of two evolutionary pathways of antigen presentation ? Seminars in Immunology 3 : 57-64, 1991.

Maki RG, Eddy RL, Byers M, Shows TB, Srivastava PK. Mapping of the genes for human endoplasmic reticular heat shock protein gp96/grp94. Somatic Cell and Molecular Genetics 19, 73-81, 1993.

Li Z, Srivastava PK. Tumor Rejection Antigen Gp96/ Grp94 is An ATPase : Implications for protein folding and antigen presentation. EMBO Journal 12 : 3143-3151, 1993.

Udono H, Srivastava PK. Heat shock protein 70 - associated peptides elicit specific cancer immunity . J Exp. Medicine 178, 1391-1396, 1993.

Blachere NE, Udono H, Janetzki S, Li Z, Heike M, Srivastava PK : Heat shock protein vaccines against cancer. J. Immunotherapy 14, 352-356, 1993.

Srivastava PK, Udono H, Blachere NE, Li Z. Heat shock proteins transfer peptides during antigen processing and CTL priming. Immunogenetics 39, 93-98, 1994

Udono H, Levey DL and Srivastava PK. Cellular requirements for tumor - specific immunity elicited by heat shock proteins : Tumor rejection antigen gp96 primes CD8+ T cells in vivo. Proc. Natl. Acad. Sci. USA 91, 3077-3081, 1994.

Heike M, Blachere NE, Wolfel T, Meyer zum Buschenfeld KH, Storkel S and Srivastava PK. Membranes activate tumor and virus-specific cytotoxic T lymphocytes in vivo and stimulate tumor-specific lymphocytes in vitro: Implications for vaccination. J.Immunotherapy 15,165 - 174, 1994.

E. ORIGINAL PEER-REVIEWED PAPERS: (CONTINUED)

Heike M, Blachere N, Srivastava PK. Protective cellular immunity against a spontaneous mammary carcinoma from ras transgenic mice. Immunobiology. 190, 411-423, 1994.

Udono H, Srivastava PK. Relative immunogenicities of Heat Shock Proteins gp96, hsp90 and hsp70 against chemically induced tumors Journal of Immunology 152, 5398-5403, 1994.

Srivastava P.K. Endo-b-D glucuronidase (heparanse) activity of heat shock protein / tumor rejection antigen gp96. The Biochemical Journal 301, 919-919, 1994.

Anderson SL, Sheng T, Lou J, Xing L, Blachere NE, Srivastava PK, Rubin BY. The endoplasmic reticular heat shock protein gp96 is transcriptionally up-regulated in interferon-treated cells. J. Experimental Medicine, 180, 1565 - 1569, 1994.

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F. BOOK CHAPTERS (SELECTED LIST): (CONTINUED)

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APPENDIX 2

Patents

U.S. Patent no. 5,750,119: Immunotherapeutic stress protein-peptide complexes against cancer. Issued May 12, 1998

U.S. Patent no. 5,830,464: Compositions and methods for the treatment and growth inhibition of cancer using heat shock/stress protein-peptide complexes in combination with adoptive immunotherapy. Issued November 3,1998

U.S. Patent no. 5,837,251: Compositions and methods using complexes of heat shock proteins and antigenic molecules for the treatment and prevention of neoplastic dis. Issued Nov. 3, 1998

U.S. Patent no. 5,935,576: Compositions and methods for the treatment and prevention of neoplastic diseases using heat shock proteins complexed with exogenous antigens. Issued August 10, 1999

U.S. Patent no. 5,948,646: Methods for preparation of vaccines against cancer comprising heat shock protein-peptide complexes. Issued September 7, 1999

U.S. Patent no. 5,961,979: Stress protein-peptide complexes as prophylactic and therapeutic vaccines against intracellular pathogens. Issued October 5, 1999

U.S. Patent no. 5,985,270: Adoptive immunotherapy using macrophages sensitized with heat shock protein-epitope complexes. Issued November 16,1999

U.S. Patent no. 5,997,873: Method of preparation of heat shock protein 70-peptide complexes. Issued December 7, 1999

U.S. Patent no. 6,007,821: Method and compositions for the treatment of autoimmune disease using heat shock proteins. Issued December 28,1999

U.S. Patent no. 6,017,540: Prevention and reatment of primary and metastatic neoplastic diseases and infectious dis. with heatshock/stress protein-peptide complexes. Issued Jan. 25, 2000

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U.S. Patent no. 6,048,530: Stress protein-peptide complexes as prophylactic and therapeutic vaccines against intracellular pathogens. Issued April 11, 2000

U.S. Patent no.6,130,087:Methods for generating cytotoxic T cells in vitro.Issued October 10, 2000

U.S. Patent no. 6,136,315: Compositions and methods using complexes of heat shock protein 70 and antigenic molecules for the treatment and prevention of neoplastic diseases. Issued October 24, 2000

U.S. Patent no. 6,139,841: Compositions and methods using complexes of heat shock protein 70 and antigenic molecules for the treatment and prevention of infectious diseases. Issued October 31, 2000

U.S. Patent no. 6,143,299: Compositions and methods using complexes of heat shock protein gp96 and antigenic molecules for the treatment and prevention of infectious diseases. Issued November 7, 2000

U.S. Patent no. 6,156,302: Adoptive immunotherapy using macrophages sensitized with heat shock protein-epitope complexes. Issued December 5, 2000

U.S. Patent no. 6,162,436:Compositions and methods using complexes of heat shock protein 90 and antigenic molecules for the treatment and prevention of neoplastic diseases. Issued December 19, 2000

U.S. Patent no. 6,168,793: Heat shock protein 70 preparations in vaccination against cancer and infectious disease. Issued January 2, 2001.

U.S. Patent no. 6,187,312: Compositions and methods using complexes of heat shock protein 90 and antigenic molecules for the treatment and prevention of infectious diseases. Issued: February 13, 2001

U.S. Patent no. 6,322,790: Compositions and methods for eliciting an immune response using heat shock/stress protein-peptide complexes in combination with adoptive immunotherapy. Issued: November 27, 2001

U.S. Patent no. 6,375,953: Treatment of infectious diseases with hsp70-peptide complexes. Issued: April 23, 2002

U.S. Patent no. 6,379,672: Prevention of infectious diseases with gp96-peptide complexes. Issued: April 30, 2002

U.S. Patent no. 6,383,491: Prevention of infectious diseases with hsp90-peptide complexes. Issued: May 7, 2002

U.S. Patent no. 6,383,492: Treatment of infectious diseases with gp96-peptide complexes. Issued: May 7, 2002

U.S. Patent no. 6,383,493: Methods and compositions for eliciting an immune response with hsp70-peptide complexes. Issued: May 7, 2002

U.S. Patent no. 6,383,494: Methods and compositions for eliciting an immune response with gp96-peptide complexes. Issued: May 7, 2002

In addition, over 50 applications are pending in the U.S.

APPENDIX 3

Major Conferences Organized

(not including sessions within conferences)

I International Conference on Heat Shock Proteins in Immune Response, October 12-15, 1998, Farmington, CT

II International Conference on Heat Shock Proteins in Immune Response, October 8-12, 2000, Farmington, CT

Winter School in Immunology, Kovalam, Kerala, India, February 5-9, 2001, Organized by Cancer Research Institute, New York

III International Conference on Heat Shock Proteins in Immune Response, October 6-9, 2002, Farmington, CT

II Winter School in Immunology, Kovalam, Kerala, India, December 16-20, 2002 Organized by Cancer Research Institute, New York

APPENDIX 4

Invited Lectures

1st International Conference on Basis & Clinical Immunogenomics, Budapest, Hungary, October 3-7, 2004
Johnson & Johnson Immune Modulation Symposium Malvern, Philadelphia, Pennsylvania, October 16, 2003
1st Annual European Conference: Perspectives in Melanoma Management, Amsterdam, The Netherlands October 9-11,
2003
CANCER VACCINES 2003-Cancer and HIV Vaccines: Shared Lessons, Manhatten, NY, October 1-3, 2003
First International Congress on Stress Responses in Biology and Medicine, Quebec City, Canada, September 10-14, 2003
International Summer School on Cancer Immunology and Immunotherapy, Ionian Village Peloponese, Greece, September
8 -13, 2003
First Annual Melanoma Research Congress, Philadelphia, PA, June 21-24, 2003
European Society of Hyperthermic Oncology ESHO2003, Munich, Germany, June 4-7, 2003
International Society for Cancer Gene Therapy Meeting, Singapore, 26th & 27th April 2003
ASCO Conference, 39th Annual Meeting, Chicago, IL, May 31-June 3, 2003
"CASC" Seminar - Rupert Sheldrake , Uconn, Farmington, CT, March 7, 2003
5th Annual Walker's Cay Collogquium on Cancer Vaccines and Immunotherapy, Abaco, Bahamas, March 5-8, 2003
Keystone Symposium, Basic Aspects of Tumor Immunology, February 17-23, 2003
American Association of Immunologists and NCI (NIH), "Research Opportunities in Cancer Immunology", Bethesda, MD,
January 22-24, 2003
Winter School in Immunology, Kovalam, Kerala, India, December 16-20, 2002
BioSecurity, 2002, Las Vegas, MGM Grand, NV, November 18-21, 2002
New England Immunology Conference (NEIC), Woods Hole, MA, November 16-17, 2002
12th New England Regional Workshop on Autoimmune Diabetes Mellitus, Woods Hole, MA, November 15, 2002
UConn Immunology Graduate Retreat, Avon Old Farms Hotel, Avon, CT, November 15, 2002
Brazilian Society of Immunology (SBI), Salvador, Brazil, October 20-23, 2002
AACR Meeting on Cancer Prevention, New York City, NY, October 14-18, 2002
EMBO, "The biology of heat shock proteins and molecular chaperones", Warsaw, Poland, September 25 -29, 2002
HSP90 Workshop, Arolla, Swiss Alps, Switzerland, August 24-28, 2002
Upenn Immunology Training Grant Retreat, Philadelphia, PA, August 1, 2002
Eurocancer 2002, Paris France, June 4-6, 2002
ASCO, Orlando, FL May 17, 2002
Annual Conference on Vaccine Research, Baltimore, MD, May 6-8, 2002
DARPA 2002 UPC PI Conference, Lexington, KY, April 6-10, 2002
World Drug Discovery Summit, Copenhagen, Denmark, April 3-5, 2002
2002 Keystone Symposia, Keystone, Colorado, February 25-March 3, 2002
Australasian Society for Immunology Conference, Canberra, Australia, 2-5 December, 2001

III International Workshop on Molecular Biology of Stress Responses, Mendoza, Argentina, October 9-13, 2001

IV "Anton Dohrn" Workshop, "New Perspectives in Tunicate Biology", Ischia, Italy, September 29 - October 2, 2001

British Society of Histocompatibility and Immunogenetics, St John's College, Cambridge, London, September 26-28,2001

"CASC" Seminar, Uconn, Farmington, CT, September 10, 2001

8thCGGH Symposium "New Paradigms of Molecular Chaperones in the Postgenome Era", August 6-9, 2001, Sapporo Japan

11th International Congress of Immunology, "Molecular interactions in infection and immunity",Stockholm, Sweden, July 22-28, 2001

International Society of Cancer Gene Therapy, IVth Mtg., London, July 12-13, 2001

Invited Lectures (continued):

Perspectives in Melanoma V: Scientific and Clinical Foundation for Future Progress, The University of Pittsburgh, June 7-8, 2001, Pittsburgh, PA

Annual meeting of the "Hinterzartener Kreis" for Cancer Research, Cadenabbia/Como, Italy, May 10-13, 2001

7th National Symposium: Basic Aspects of Vaccines, Baltimore, MD, May 2-4, 2001

Winter School in Immunology, Kovalam, Kerala, India, February 8-13,2001

Keystone Symposia on Molecular and Cellular Biology, "Interfaces between innate and adaptive immunity, Keystone, CO, January 22-27, 2001

The British Society for Immunology Congress 2000, "Heat shock proteins: The fountainhead of innate and adaptive immune responses", London, December 5-8, 2000

University of London, Guy's King's & St. Thomas' Medical School, Annual Immunobiology Research Day, London, December 4, 2000

Dana Farber Cancer Institute, "International Symposium on Heat Shock Proteins in Biology and Medicine, Woods Hole, MA, November 6-8, 2000

II International Conf. on Heat Shock Proteins in Immune Response, Farmington, CT, October 8-12, 2000

Cancer Research Institute, Cancer Vaccines 2000, New York City, NY, October 2-4, 2000

EFIS 2000, European Federation of Immunological Societies, 14th European Immunology Meeting, Poznan, Poland, September 23-27, 2000

EFIS 2000, Heat Shock Proteins: Immune, Stress response and apoptosis, Gdansk, Poland, September 20-22, 2000

4th EFIS Tatra Immunology Conference, "Molecular Determinants of T Cell Immunity", Tatra Mountains, Slovakia, September 2-7, 2000

Proceedings of Millennium Second World Congress on Vaccines and Immunization, Liege, Belgium, August 29-September 2, 2000

University of CT Cancer Symposium, University of CT, Farmington, CT, May 11, 2000

DARPA 2000 BWD UPC/AD Conference, Fort Lauderdale, FL, January 30, 2000 - February 1, 2000

Annual Meeting of the Japanese Society for Immunology, Nagasaki University, Japan, November 28 - December 4, 1999

1st Annual Pathogenesis Symposium, University of CT, Farmington, CT, November 4, 1999

Drug Discovery for 21st Century, Worcester, MA, October 28, 1999

Cancer Research Institute, "Cancer Immunosurveillance", New York City, NY, October 4-6, 1999
1999 Annual Meeting, New England Surgical Society, Newport, RI, September 24-26, 1999
NIH - EI Study Section, Washington DC, June 17-18, 1999
Symposium of the SFB 432, "Immunological Mechanisms of Tumor Defense", Johannes Gutenberg-Universitat Mainz Klinikum, Mainz, Germany, February 26-27, 1999
Clinical Evaluation of 2nd Generation Cancer Vaccines, London, UK, February 24-25, 1999
DARPA Conference for Unconventional Pathogen Countermeasures, Monterey, CA, February 8-12, 1999
NMHCC Bio/Technology Division's Conference, Washington, DC, November 19-20, 1998
Robert Steel Foundation International Symposium 1998, Memorial Sloan-Kettering, "New Strategies for Stimulating and Augmenting Host Resistance to Malignant Cells", New York, NY, October 21-23, 1998
Cancer Research Institute Symposium, Cancer Vaccine Week 1998, New York City, New York, October 5-9,1998
AACR "Cellular Targets of Viral Carcinogenesis", Dana Point, California, September 24-28, 1998
Invited Lectures (continued):

National Cancer Institute, "Mechanisms of Immune Evasion by Tumors", Washington, DC, September 1-2, 1998
American Cancer Society 13th Annual Excalibur Round Table, Greenwich, CT August 13-14, 1998 Pfizer Mini-Symposium, "Novel Methods for Enhancement of Immune Responses to Peptides/Proteins", Groton, CT, July 24, 1998
2nd International Cancer Immunotherapy and Gene Therapy Conference, NMHCC Bio/Technology Conference Division, Waltham, Massachusetts, CT, June 15-16, 1998
Molecular Chaperones in Biology & Medicine, Mosbach, Germany, April 2-4, 1998
Symposium on Graft-versus-Host & Graft-versus-Leukemia Reactions 1998, Munich, Germany, March 28, 1998
Miami Nature Biotechnology Winter Symposia, Miami, FL, February 7-11, 1998
Immunological Attacks on Cancer, Cold Spring Harbor Laboratory, NY, October 19-22, 1997
International Workshop on Molecular Biology of Stress Response, Benares Hindu University, Varanasi, India, October 12-17, 1997
1997 Fourth Annual CapCURE Scientific Retreat, Lake Tahoe, Nevada, September 4-7, 1997
Molecular Virology and Vaccinology meeting of Drug Information Association, April 9-12, 1997, Newport Beach, California
UCLA symposium on "Cancer Immunity and Immunotherapy", Copper Mountain, Colorado, February 1997
Annual Meeting, American Society for Histocompatibility and Immunogenetics, San Diego, California, December 1996
Speaker and Organizing Committee member, Cancer Vaccines 1996, Cancer Research Institute, New York, October 1996
Plenary Speaker, Annual Meeting of German Society of Immunology, Hamburg, Germany, September 1996
European Immunology (ENII) meeting, Les Embiez, France, May 1996
Cancer Vaccines Conference (Henry Stewart Conference Studies), London, England, May 1996
Symposium in Immunology VI, Tumor Immunology, Organized by Immuno Pharma, Prague, Czechoslovakia, March 1996
European Heat Shock Meeting, Berlin, Germany, February-March 1996
Plenary Speaker, Annual Meeting of Canadian Society of Immunology, Montreal, Canada, March 1996
Plenary speaker, Annual Meeting of British Society of Histocompatibility and Immunogenetics, Liverpool, England, February 1996

Symposium speaker on cancer Vaccines, Annual Meeting, American Association of Advancement of Sciences, Baltimore, MD, February 1996

First International Workshop on Antigen Presentation, Oxnard, California, November 1995

Nobel Forum conference on Cancer Immunity, Stockholm, Sweden, September 21-23, 1995

Symposium speaker, IX International Congress of Immunology, 1995, San Francisco, July 1995

II International Conference on Engineered Vaccines against Cancer and AIDS, San Francisco, March 3-5, 1995

Deutsches Forschungsgemeinschaft, DFG workshop on Immunological aspects of heat shock proteins and heat shock response, December 1994

International Conference on "Cancer Vaccines", Cancer Research Inst., New York, October, 1994.

Distinguished Lecturer, University of Alabama, September 1994

IBC Conference on "Therapeutic Opportunities for Heat Shock Proteins", Cambridge, MA; September 29-30,1994

Invited Lectures (continued):

Johns Hopkins Oncology Center course on "New Approaches to Cancer Therapy", Baltimore, May 16, 1994

AACR Annual meeting, Symposium on "New Approaches to Cancer Immunotherapy", San Francisco, April 1994

Cold Spring Harbor Meeting on the 'Biology of heat shock proteins and molecular chaperones', May 4-8, 1994

German Society for Cell Biology meeting, Lbeck, Germany, March 1994

US - Japan Bionational Immunology Symposium, Bethesda, MD January 1994

Mildred Scheel Lecturer in the conference "Hyperthermia in Clinical Oncology", Ludwig Maximilians, University of Munich, Germany, November 1993

FASEB Conference on Tumor Immunotherapy, Vermont, June 9, 1993,

Workshop on "Heat shock in Multiple Sclerosis and other disorders", Galicia, Spain, MS Society, April 2-5, 1993

UCLA symposium on Cellular Immunity to Cancer, Taos, New Mexico, March 1993

American Association of Immunologists meeting, Anaheim, CA, Session on Tumor Antigens, April 1992

Plenary speaker, Annual Meeting of Austrian Society for Allergology and Immunology, Graz, May 1991

Chairman, Symposium on "Nature of Tumor Antigens" at the American Association of Immunologists meeting, New Orleans, Lousiana, June 1990

Special AACR symposium on 'Molecular Basis of Tumor Immunology', Virginia, May 20-22 1990

UCLA Symposium on 'T Cell Immunity to Cancers' January - February 1990

VII Int'l Congress of Immunology, Berlin, Workshop on Tumor Antigens, 1989

NIH workshop on 'Influence of MHC Expression on Tumor Growth' Annapolis, Maryland, 1988

UCLA Symposium on 'Human Tumor Antigens and Specific Tumor Therapy', 1988

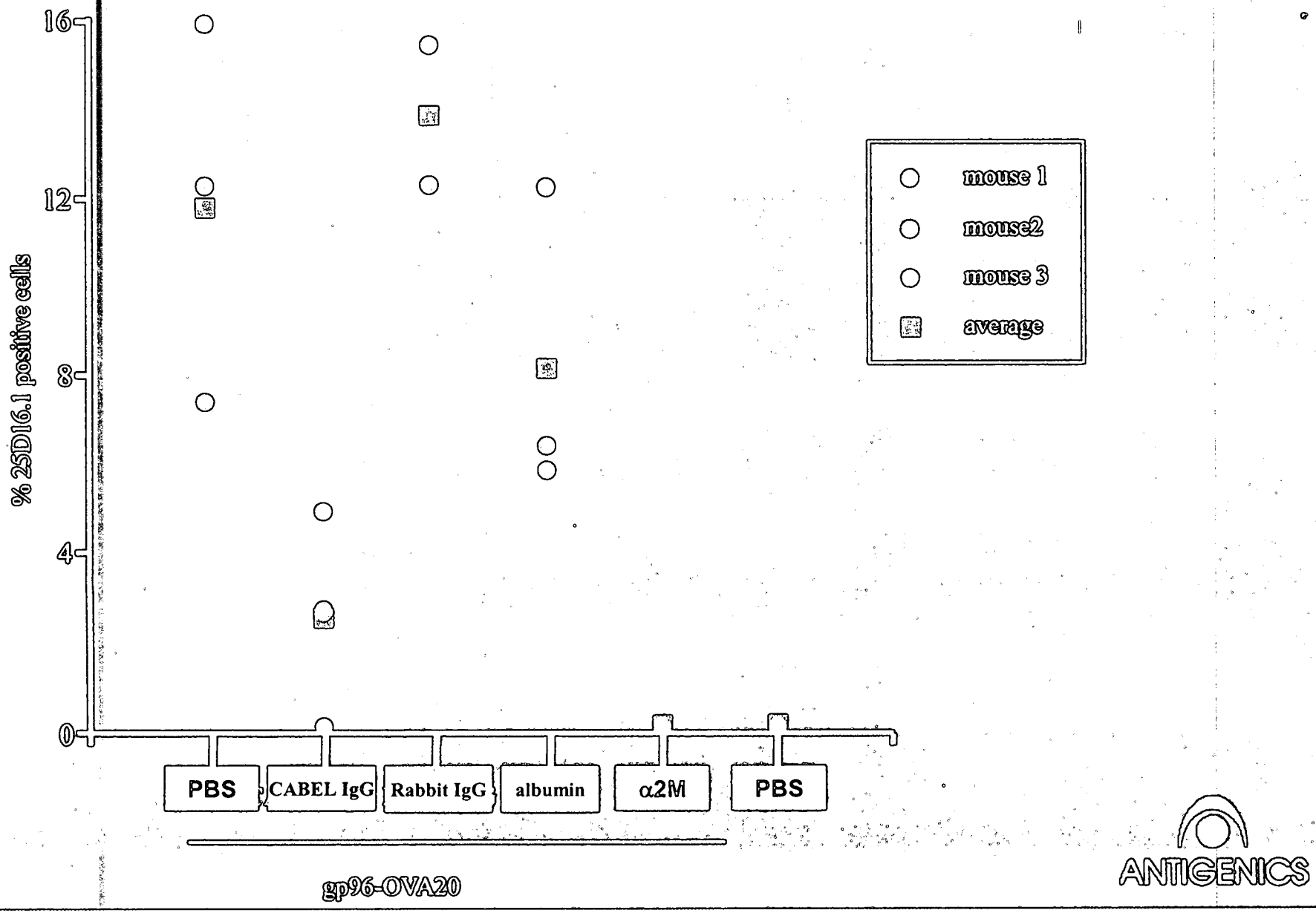
Cancer Research Institute workshop on 'Recent Advances in Human Melanoma Research'; New York, 1987

Also gave invited talks at:

Duke University, Emory University, New York University, Cornell University Medical School, Johns Hopkins University, German Cancer Research Center (Deutsches Krebsforschungszentrum), Heidelberg, Yale University, Sloan-Kettering Institute, University of Mainz (Germany), Max - Planck Institut at Freiburg, Germany, Ludwig Institute for Cancer Research, Stockholm, Sweden, Netherlands Cancer Institute,

Amsterdam, Holland, Center for Cellular and Molecular Biology, Hyderabad, India, Albert Einstein School of Medicine, New York, National Cancer Institute, NIH, The Jackson laboratory, Bar Harbor, Chiron Corporation, University of Texas, Houston, TX., Washington University, St. Louis, MO., MD Anderson Cancer Center, Houston, TX., Boehringer-Manheim, Penzburg, Germany, University of Connecticut, Farmington, Sigma-Tau Company, Rome, Italy, University of Lund, Sweden; Stanford University, Palo Alto, University of California at Berkley, University of Pennsylvania, Pennsylvania State University, Oxford University, England; Michigan Cancer Foundation, Detroit, Michigan; Mayo Clinic, Rochester, MN; University of Southern California; University of California at Norris; University of Tennessee, Cornell University, New York; Massachusetts General, Harvard Medical School, New York; Karmanos Cancer Institute, Detroit, Michigan; Boehringer Ingelheim, Ridgefield, Connecticut; University of Rochester, Rochester, New York; Roswell Park Cancer Inst. , Buffalo,NY; Entremed Inc. ,Rockville, Maryland; Elan Pharmaceuicals, South San Francisco, California; University of Connecticut, Storrs, Connecticut; Stazione Zoologica Anton Dohrn, Naples, Italy; Fox Chase Cancer Ctr, Pennsylvania; Cleveland Ohio State Univ., Ohio; UCSF Cancer Ctr, San Francisco, California; Rockefeller University Hospital, New York City, NY; Wistar Institute, Philadelphia, PA; Case Western, Cleveland Ohio; University of Leeds, London; NCBS, Bombay, India; Baylor University, Houston, TX; Indian Institute of Science Bangalore, India; University of CT, CASC Series, Farmington, CT; University of Michigan, Ann Arbor, MI; Sequella Foundation, Rockville, MD; Johns Hopkins Univ, Baltimore MD; PTC Therapeutics, Plainfield, NJ; UMDNJ-New Jersey Medical Center, NJ; Medical College of Wisconsin, Milwaukee; University of Pittsburgh Cancer Institute, PA; Indiana University School of Medicine, Indianapolis, IN; Scripps Research Institute, La Jolla, CA; University of Toronto, Ontario, Canada;

FIGURE 1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PBS
CABEL IgG
Rabbit IgG
albumin
α2M
PBS
gp96-OVA20
ANTIGENICS

FIGURE 3

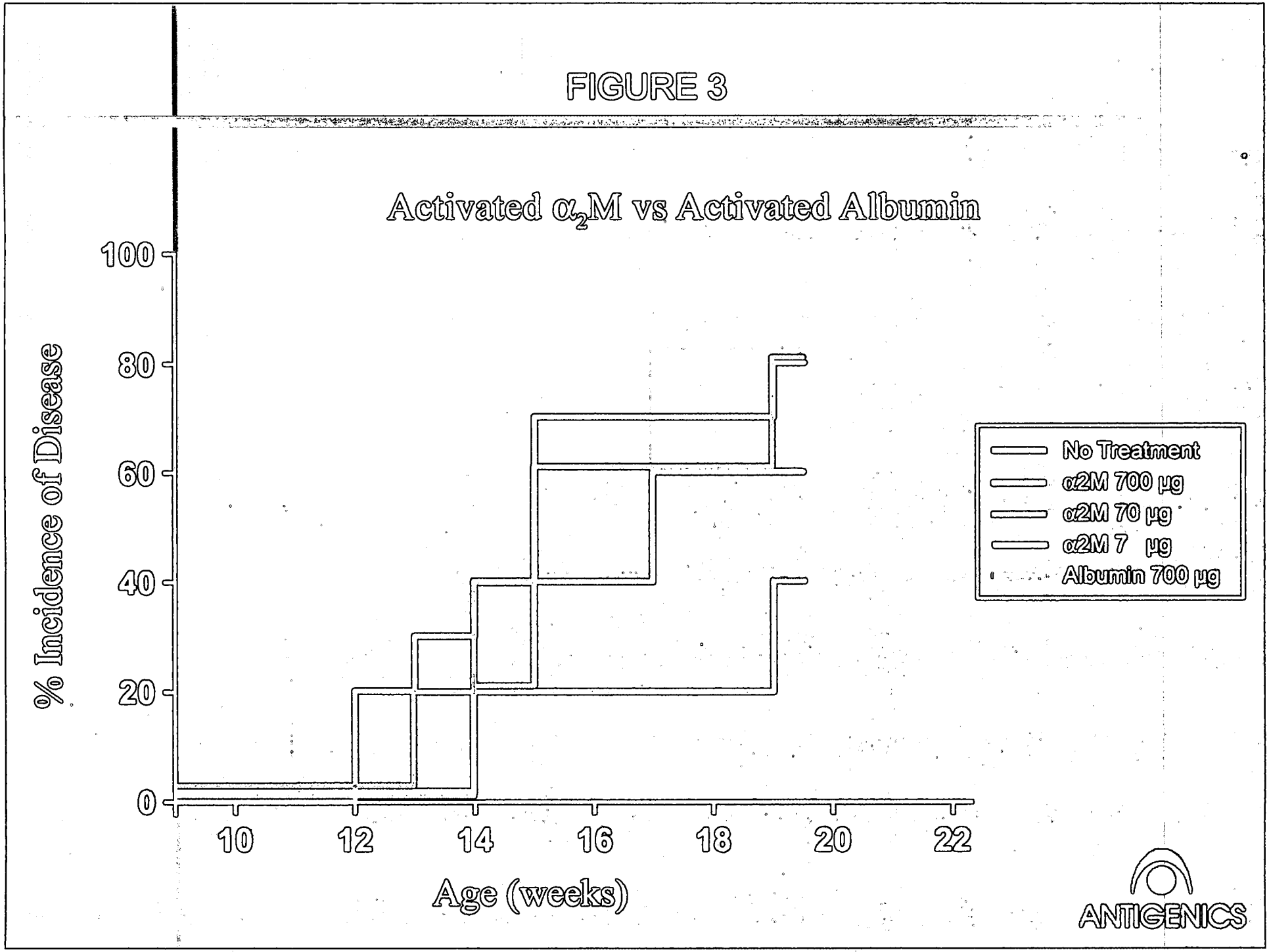
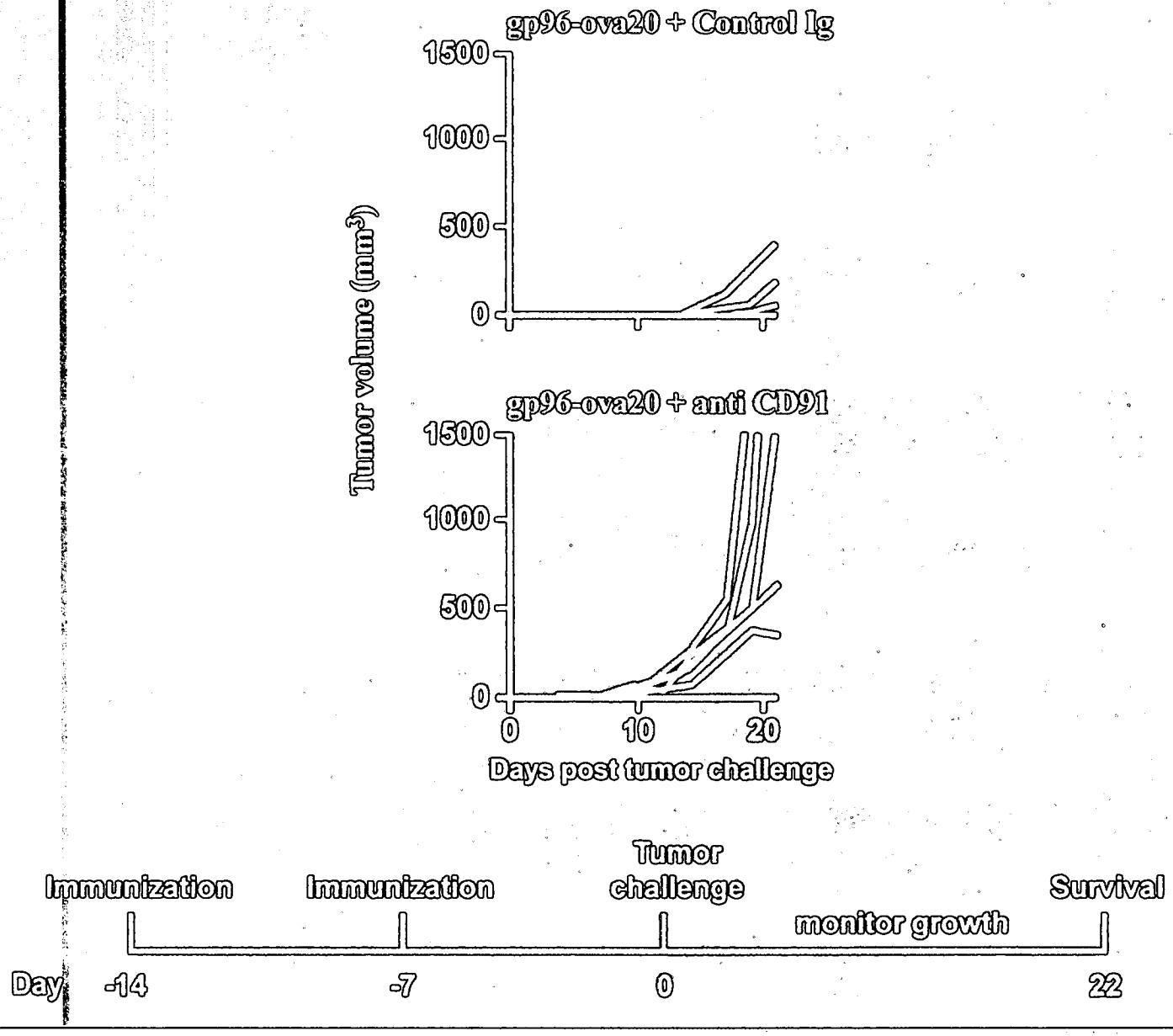

Activated α_2M vs Activated Albumin
% Incidence of Disease
100
80
60
40
20
0
10
12
14
16
18
20
22
Age (weeks)
No Treatment
α2M 700 µg
α2M 70 µg
α2M 7 µg
Albumin 700 µg
ANTIGENICS

FIGURE 2


gp96-ova20 + Control Ig
1500
1000
500
0
Tumor volume (mm³)
gp96-ova20 + anti CD91
1500
1000
500
0
0
10
20
Days post tumor challenge
Immunization
Immunization
Tumor challenge
Survival
monitor growth
Day
-14
-7
0
22
ANTIGENICS

APPENDIX 3

MATERIALS AND METHODS FOR EXPERIMENTS

In Vivo Re-Presentation Assays

Gp96 was prepared as described in US Patent no. 5,750,119. The OVA20-mer peptide (NH2-SGLEQLE**SIINFEKL**TEWTS-COOH) was synthesized by Genemed (CA) to >95% purity. To prepare gp96-peptide complexes, gp96 was heated to 50°C in the presence of 50 molar excess of OVA 20 peptide. The complexes were placed at room temperature for 30 min and then placed on ice. Free, uncomplexed peptide was removed using a Centricon 50 (Millipore). Complexes thus made were used for immunizations.

For alpha (2) macroglobulin purification, serum from mice was diluted 1:1 with 0.04 M Tris pH 7.6, 0.15 M NaCl and applied to a 65 ml Sephacryl S 300R (Sigma) column equilibrated and eluted with the same buffer. Alpha (2) macroglobulin-positive fractions were determined by a dot blot and the buffer changed to a 0.01 M sodium phosphate buffer pH 7.5. The protein was applied to a concanavalin A sepharose column. Bound protein was eluted with 0.2 M methylmannose pyranoside and applied to a DEAE column equilibrated with 0.05 M sodium acetate buffer. Alpha (2) macroglobulin was eluted in a pure form with 0.13 M sodium acetate buffer and analyzed by SDS-PAGE and immunoblotting. Some experiments were carried out with alpha (2) macroglobulin purchased from Sigma.

Protein peptide complexes were administered to C57BL/6 mice (Jackson Labs, Bar Harbor, ME) at 6-8 weeks of age by intradermal and ventral injections. This allowed re-presentation of chaperoned peptides to occur, culminating in the presentation of peptides by dendritic cells of the lymph nodes.

This *in vivo* re-presentation of peptides was measured using an antibody specific for SIINFEKL/K^b complexes (25D.1-16). Previous work has shown that injection of 1μg of gp96 complexed to OVA20, intradermally, led to detectable staining of dendritic cells in the lymph nodes.

Injection protocols (5 mice per group):
1ug gp96-OVA20 + 50ug CABEL
1ug gp96-OVA20 + 50ug control rabbit IgG

1ug gp96-OVA20 + 50ug alpha (2) macroglobulin
1ug gp96-OVA20 + 50ug albumin (mouse)
1ug gp96-OVA20 + 50ug PBS

Inguinal lymph nodes were removed after 6 hours, stained with 25D.1-16 and analyzed by FACS. The percent 25D.1-16 positive cells was measured (yellow circles in Figure 1) and an average percent (blue squares in Figure 1) was calculated for the percent values of each column of data points which correspond to the test compounds administered.

Tumor Challenge Assays

Protein peptide complexes were prepared as described above. C57BL/6 mice (Jackson labs, Bar Harbor, ME) at 6-8 weeks of age were injected on days -14 and -7 with 100μg of anti-CD91 antibody or control Rabbit IgG in the presence of one μg of gp96-peptide complex. All injections were done intradermally in 100μl PBS. Further 100 μg of antibody was injected at the same site on days -13, -12, -6 and -5. Live B16-F10-OVA tumor cells (100,000) were washed free of culture medium, resuspended in PBS and injected intradermally one week after the last immunization (day 0). Tumors were measured in 2 dimensions. Half of the average was used as the radius of the tumor to calculate the tumor volume. P values were determined using single-classification analysis of variance (ANOVA).

NOD Mouse Model Assays

In vivo assays of efficacy of the treatment regimens were assessed in female NOD/LtJ mice (commercially available from the Jackson Laboratory, Bar Harbor, ME). NOD mice develop diabetes as a result of auto reactive T cell destruction of the beta cells of the pancreas. Incidence rates of diabetes in these mice were consistent with those reported in literature, wherein over 80% of female mice developed diabetes by 24 weeks of age and onset of insulitis commenced between 6-8 weeks of age. NOD mice are inbred and highly responsive to a variety of immunoregulatory strategies. Adult NOD mice (6-8 weeks of age) have an average mass of 20-25 g. Female NOD/LtJ mice 9 weeks of age were used in all of the assays.

To test the effectiveness of CD91 antagonists, such as alpha-2-macroglobulin, as a therapy for treating autoimmune disease, mice were treated with either 7.0 μg, 70.0 μg, or 700.0 μg of activated alpha-2-macroglobulin, or 700.0 μg of activated mouse albumin. Two

injections, one week apart, at week 9 and week 10, were administered subcutaneously under the dorsal skin of each mouse.

Monitoring was performed prior to immunization and performed weekly throughout the treatment and continued thereafter. Urine was tested for glucose every week (Diastix TM; Fischer). The monitoring portion of the experiments can also be preformed by checking glycosuric mice for serum glucose (ExacTechTM, MediSense, Inc., Waltham, MA). The percent incidence of disease was calculated using urine glucose levels and plotted against age of NOD mice, see Figure 3.